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Our goal was to determine whether protein kinase C (PKC) activation caused a redistribution of PKC in smooth muscle. To accomplish this, we examined the three-dimensional distribution of PKC in smooth muscle before and after cell activation. Smooth muscle cells were enzymatically dispersed from the toad stomach. Bufo marinus, adhered to coverslips and treated with either 10 μM carbachol, 1 μM phorbol 12-myristate 13-acetate (PMA), 1 μM 4-α phorbol 12,13-didecanoate (4-α PMA, an inactive phorbol ester) or amphibian physiological saline. Cells were then fixed with paraformaldehyde, permeabilized with triton X-100 and immunofluorescently labelled for PKC and vinculin. Vinculin was labelled to identify the cell surface and cell membrane contractile filament anchorage sites. The anti-PKC IgM was capable of immunoprecipitating PKC from a PKC containing reaction mixture. The immunoprecipitation of PKC was found to increase in a dose-dependent manner by adding PMA to the reaction mixture. This indicated that the anti-PKC used for immunolocalization preferentially recognized the activated form of PKC. Fluorescent images of labelled cells were collected as a through focus series of two-dimensional images spaced at 0.25 μm intervals using a digital imaging microscope. These images were subjected to a deblurring image restoration program using a constrained deconvolution algorithm. The restored images were reconstructed into three-dimensional displays of the intracellular distribution of PKC and vinculin. At rest, PKC and vinculin were located at the cell periphery and organized longitudinally into roughly parallel strands. At the cell periphery, 18% of the PKC colocalized with vinculin. In the cell interior, labelling for PKC was sparse and diffuse. Following carbachol, PKC exhibited a more pronounced and strand-like cytosolic distribution. Also, there was an increase in the total fluorescence intensity of PKC labelling. Treatment with PMA but not 4-α PMA produced similar changes in PKC distribution. These results indicate that in the resting smooth muscle cell there is a pool of activated PKC near the cell membrane and that after stimulation the activated PKC is not confined to the cell membrane but can be observed throughout the cytosol. In the cytosol, the apparent association of PKC with contractile filaments suggests a role for PKC in modulatory contractile function.

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G-PROTEIN DEPENDENT ENHANCEMENT OF VASCULAR SMOOTH MUSCLE MYOFILAMENT Ca^{2+} SENSITIVITY

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In smooth muscle, force development is more sensitive to Ca^{2+} during agonist stimulation than during membrane depolarization. We used alpha-toxin permeabilized smooth muscle to investigate the mechanism(s) of the enhanced Ca^{2+} sensitivity. This preparation retains receptor-coupled events while allowing control of the intracellular environment. Stimulation of alpha-toxin permeabilized rabbit mesenteric arteries with 0.3 μM Ca^{2+} induced a monotonic increase in force and myosin light chain (MLC) phosphorylation. The inclusion of 10 μM norepinephrine (NE) and 10 μM GTP resulted in the monotonic increase in force to significantly greater levels. However, MLC phosphorylation levels only transiently increased to higher levels in response to NE plus GTP, then fell with time to values similar to those in response to Ca^{2+} alone. Exposure of the permeabilized arteries to inhibitors of protein kinase C (PKC) specifically abolished the enhanced force and transient increase in MLC phosphorylation in response to NE plus GTP. These data suggest that receptor dependent PKC activation inhibits MLC phosphatase activity, possibly by PKC catalyzed phosphorylation of a MLC phosphatase inhibitor. In addition, there must be a second process that maintains the agonist induced enhanced force at MLC phosphorylation levels similar to those in response to Ca^{2+} alone. This study was supported in part by NIH grants HL37956 and HL46704.

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TWO DISTINCT α_1-ADRENOCEPTOR SUBTYPES IN RABBIT THORACIC AORTA

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α_1-Adrenoceptors are not homogeneous in all tissues and their heterogeneity has been suggested to be related in part to the presence of different α_1-adrenoceptor subtypes. In the rabbit thoracic aorta, noradrenaline with a very wide range of concentrations (from 1 nM to 100 μM) produced a concentration-dependent contraction. The concentration-response curve was antagonized by prazosin, resulting in a rightward displacement of the curve. However, the shift was not proportional to the concentration of prazosin; Schild plots showed that the inhibition by prazosin was biphasic, implying that noradrenaline acted through two receptor populations (pK_B = 10.0 and 8.8). However, in the strips pretreated with chloroethylclonidine (CEC; an α_{1B} inactivating agent), noradrenaline with a narrow range of concentrations (from 30 nM to 100 μM) produced a concentration-response curve, which was competitively inhibited by prazosin (pK_B = 8.3). ^{3}H-prazosin bound to two distinct populations of α_1-adrenoceptors of the aortic membranes (pK_D = 9.94 and 8.59). Pretreatment with CEC abolished the 3H-prazosin binding to the prazosin-high but not -low affinity sites. Noradrenaline showed slightly higher affinity for the prazosin-high affinity site than for the low affinity site. From these results, it is suggested that in the rabbit thoracic aorta there are two distinct α_1-adrenoceptor subtypes

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